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BONE AS A PHYSIOLOGICAL TOOL*

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I: INTRODUCTION

I will spend little time telling you what we have done and how, because I believe you will be more interested in *why*.

Regarded apart from the cells that actually inhabit and festoon it, bone is an extracellular substance with remarkable properties. Among them are these five: (*i*) Bone is a direct product and measure of cell activity. (*ii*) Once made, its structure and composition are stable for years. (*iii*) Both structure and composition contain information about the cell behavior that made it. (*iv*) Therefore bone is a record of the past behavior of its cells.[10] (*v*) The record is read *after* it is "written in" so that *in vivo* human cell behavior can be studied with assurance that the act of observation will not disturb it.[8]

We can read this record largely because of four things that took place in the last 20 years: (*i*) The ancestry of cells and of cell populations can now be traced by tritiated thymidine labelling.[3,19,20,24] (*ii*) There are now simple ways of making large numbers of undecalcified bone section[5,7,23] (see figure 1). (*iii*) The tetracycline antibiotics deposit in, and label newly forming bone *in vivo,* and so are a tissue time marker[14,18] (See figure 2). (*iv*) Simple methods of quantitative histological analysis now let us get accurate data quickly from a large amount of material.[10,16]

II: ONE WAY OF RELATING CELLS TO PHYSIOLOGY

With these and other aids, three things have been learned about the role of cells in human physiology. If they are as important as they now seem, they will lead to modification of some of our present ideas.[9,10]

These things are:

1) The chain of cell responsibility in human physiology has two different links, each an essential class of cell population: (*i*) proliferating, undifferentiated cells, and (*ii*) nonproliferating, differentiated cells.[17] In contrast, unicellular organisms and even the lower metazoa have no such subdivision[8] (See figure 3).

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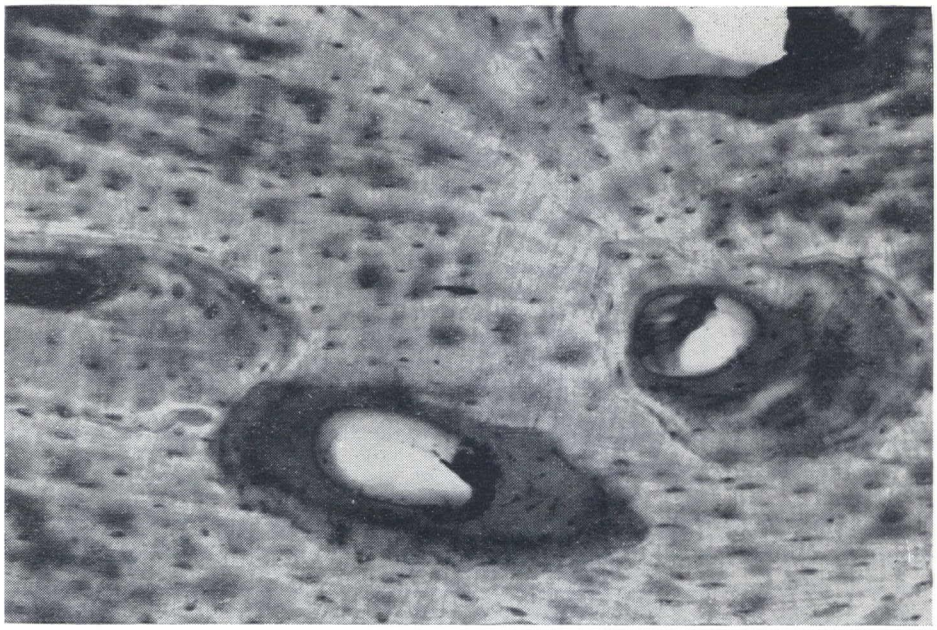

Figure 1

Cross section fibula, 100X, mineralized section, basic fuchsin, from a 9-year-old boy with vitamin D resistant rickets. The photomicrograph shows several osteoid seams. It also shows the pattern of "halo volume" permeability (i.e., perilacunar failure to mineralize adequately) which seems to be diagnostic of this disease. Case submitted courtesy B. Frame, M.D. and J. Fleming, M.D.

2) A) *The Proliferating Cells* (i.e., stem cells, *mesenchymal cells,* reticulum cells, pluripotent cells) do no metabolic "work" for the rest of the body. Thus, if all proliferating cells were to be suddenly removed from the body, there would be no immediate effect on one's health.[15] In adults, less than one cell in 200 is a mesenchymal cell. The job of proliferating cells is to make new daughter cells,[24] which are of two kinds: (A) copies of the parent cell, and (B) specialized cells. Numerically speaking, most cells in the body have life spans much shorter than a man's, so new supplies of cells are constantly needed to keep us going. Hence, one need for progenitor cells.

B) *Metabolically Specialized Cells* do jobs needed by the rest of the body to sustain life from moment to moment. Examples of such cells are (*i*) epithelial cells of the skin, (*ii*) neurons, (*iii*) cardiac muscle fibers, (*iv*) renal tubular cells, and (*v*) erythrocytes. Their jobs are respectively: (*i*) mechanical, thermal and osmotic protection of the body from the outside world; (*ii*) coordination, message reception and generation; (*iii*) pumping blood; (*iv*) clearing the blood of chemical wastes; and (*v*) carrying oxygen and carbon dioxide. Clearly, were any of these subtracted as a class from the body, we would soon die.

Metabolically specialized cells do not divide, in effect and probably in actuality,[17] and so cannot replace themselves.* Thus, functionally, one class of cells keeps us

*The changes that occur in repair are specifically *not* included in this statement.

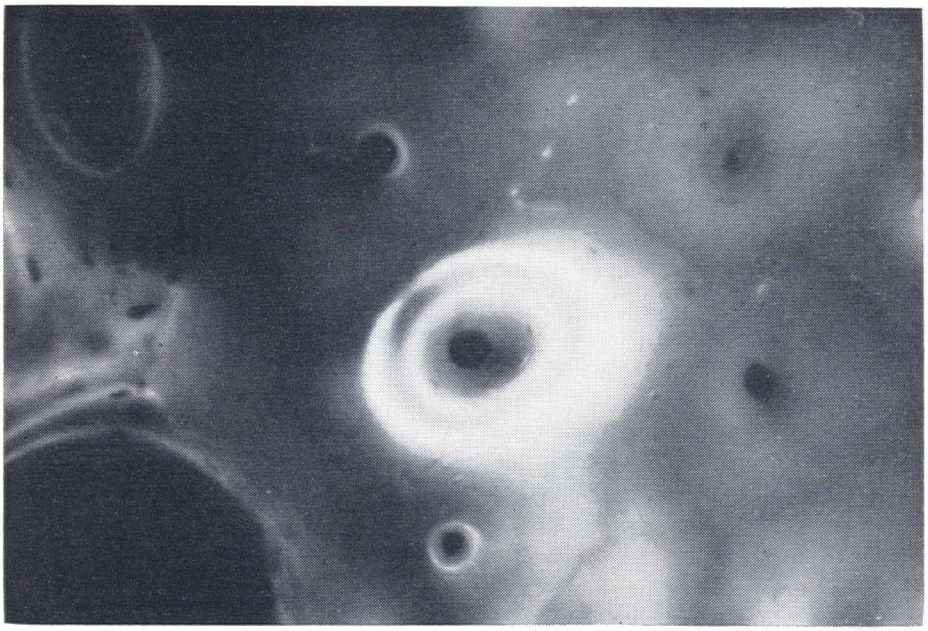

Figure 2

Blue-light fluorescence microscopy, mineralized cross section of a dog's rib, treated with fluoride. About 300X, the bright rings are two tetracycline labels given four months before the animal was sacrificed. The labels are like growth rings in trees. Part of a study supported by the Upjohn Co., with the cooperation of R. Johnston, D.V.M.

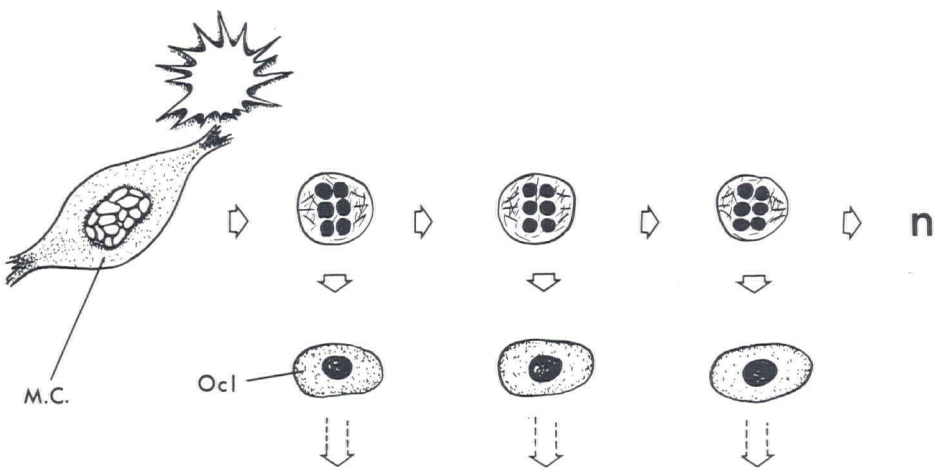

Figure 3

A diagram of the division of cellular labor in man. A mesenchymal cell, M.C. (i.e., progenitor cell) makes new cells through cell division. The specialized cells, here osteoclasts, Ocl., do the work needed by the rest of the body for survival, but they are short lived. The mesenchymal cells keep replenishing them and thus continue the functional and genetic line.

alive from moment to moment, while its deceased members are replaced by another class of cells.

3) *Systems*: In every organ and tissue in the body, cells are used in various ways as building blocks to make cell systems, which in turn are used as building blocks to make supersystems, and so on. In this way an organizational pyramid is made, and known as an organ or a person. Each level creates new and simple functions, nonexistent in lower systems in this pyramid, nor in individual cells, nor in individual biochemical pathways; each is essential to understanding the man, just as the transmission and ignition system are to the working of a car. Each of these levels is related to a cell in a man as the transmission and carburetion are to the physics and chemistry of an iron atom in a car.

This system's place in the scheme of organization is really quite upsetting to the simple idea that the keys to disease lie in understanding enzymes, or the structure of DNA. For even a complete knowledge of the cell would not *alone* allow us to understand our own physiology.[1] The proof of this is mathematical and rigorous but it can be illustrated with one practical example: Given a complete knowledge of the cell, predict on this basis *alone,* the emotion, love. It can't be done, because it is one of an infinite number of possible predictions.

Significantly, however, while we can't use molecular biology to *predict* physiology and disease, it must be invoked to explain life and disease as we find them to exist. In other words, *we can use molecular biological knowledge of cells to understand disease we already recognize, but not to predict disease we are still unaware of.* See figure 4.

The three things mentioned above are essential for true understanding of disease, although not alone sufficient for it. With respect to the "car of life", scientists are expending an enormous amount of effort on the chemistry and physics of the iron atom, but much, much less on recognizing and understanding its higher levels of organization such as the suspension, carburetion, electrical and steerage mechanisms.

III: Bone As a Research Tool

Having set a frame of reference, bone comes on stage thus:

As a special example of organs in general, bone lets us see and measure the behavior of its different populations of progenitor and specialized cells under a wide range of conditions, and it lets us identify and study the structure of the cellular organization that makes and maintains the skeleton.[10] Thus bone is a tool for doing physiological research.

In the physiology of bone, with its chemical regulation and mechanical relationship, with the effect of physical usage and age, with remodeling, with repair, with nutrition, with growth and with disease, the roles of the information in the genetic

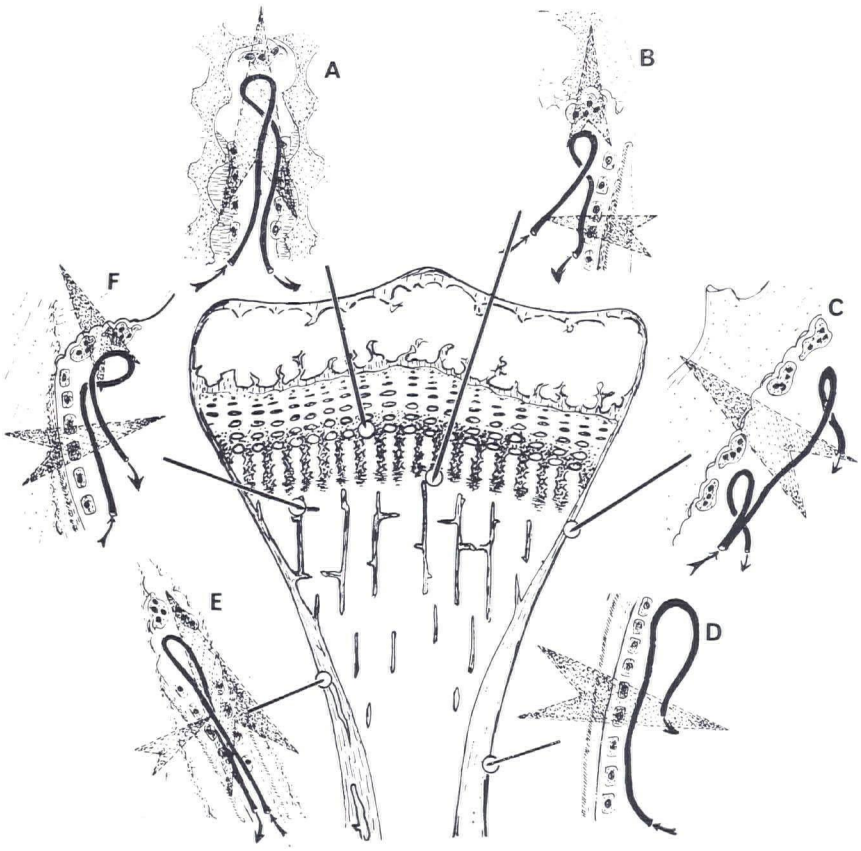

Figure 4

The upper end of a growing tibia in diagram. The tibia (and any other bone) is a *system,* made over time by a group of subsystems, six of which are indicated here. They are controlled so that they "turn on" and "turn off" at precise times and in specified places and in irreversible order in time. This control establishes the finished bone, and it is the "program" that operates the pool of genetic information in the cell. This program is just as essential in understanding physiology and disease as is the DNA encoded information in cell nuclei.

pool can all be studied and measured *in vivo*. Thus, bone is one of the most suitable tissues in which to study such phenomena as they operate in man.

When this work began in Buffalo in 1953, I hoped that the kinds of cell dynamic changes in bone disease (such as Cushing's syndrome) might parallel those in at least some of the body's soft tissues. This would make bone research much more interesting, because it would let us measure, in bone, indices of cell functions that cannot be studied safely in the soft tissues of a living man. There is now substantial evidence that the cell population kinetics in bone parallel those in many soft tissues in many diseases. Furthermore, these kinetics are often essential, and sometimes also sufficient, to cause the changes that we recognize as disease. Cell population dynamics are hard to measure in animals which can be sacrificed; in man's tissues I believe

they cannot be studied at present on any volume basis except in bone. Even here practical problems slow us down in delivering this information. As a result, bone* is a powerful research tool in studying human cell population kinetics and its disorders. However, this tool is not for routine diagnosis and follow-up, except in metabolic bone disease, where it excels all other available ways of defining the cellular disturbance in tissue.

IV: Some Successes

It took about 12 years to make and sharpen this tool. It is just now being used systematically to study disease, mechanisms of drug and endocrine effect on cell population kinetics, and the like.

With this tool and with the assistance of Dr. F. Whitehouse and Dr. J. Bryan of the Division of Metabolism, we[25] have shown clearly that: (*i*) Insulin and tolbutamide are not complete treatments for diabetes mellitus;[14] (*ii*) A disturbance in cell population kinetics in diabetes is not corrected by insulin;[14] (*iii*) A different cell population kinetic disturbance occurs in rheumatoid arthritis. (This study continues under the supervision and active participation of Dr. Howard Duncan.[4]) (*iv*) A major action of hormones in man is on cell population kinetics,[16] and effect that seems at least as important as their biochemical effects with which the literature is preoccupied at present. (*v*) In cooperation with Doctors B. Frame and R. Smith of the Ford Hospital Medical Staff, and L. Hurxthal and D. Baylink of the Lahey Clinic,[11] we have shown, as many suspected, that the osteoporoses and osteomalacias are groups of diseases needing further analysis to be understood. (*vi*) Serum chemistries and other standard laboratory tests are unreliable in detecting and classifying metabolic bone disease, because these diseases are peculiarly dynamic, and only with a tissue marker can the cell dynamics be clearly defined. (*vii*) The transverse growth and geometry of bone, i.e., its gross anatomy, is controlled by a simple negative feedback system which uses biomechanical factors to control *patterns* of cell behavior.[6,9] This control is beautifully exact and simple in essence, and has been stated in precise mathematical terms which can predict the bone effects of changes in function. See figure 5.

As we learn more, probably easier ways of studying cell and tissue dynamics will be developed. But bone will remain an indispensable research tool, because it gives us a direct and reliable way of measuring and studying certain elusive kinds of cell behavior in man, combined with complete assurance that this study cannot affect the cells because what they do happens before it is measured.

Our work has grown out of the developmental stage and moved into a phase of systematic study of cell population kinetics in various human diseases.† It has been richly rewarding, although it has presented us with a formidable and still only partially solved problem of how to communicate our data and their meanings to our audience.

*As it has been used in the Henry Ford Hospital Orthopaedic Research Laboratory.
†On Cushing's syndrome; thyrotoxicosis; diabetes mellitus; rheumatoid arthritis; physiological aging; osteoporosis; hyperparathyroidism; osteomalacia; osteogenesis imperfecta.

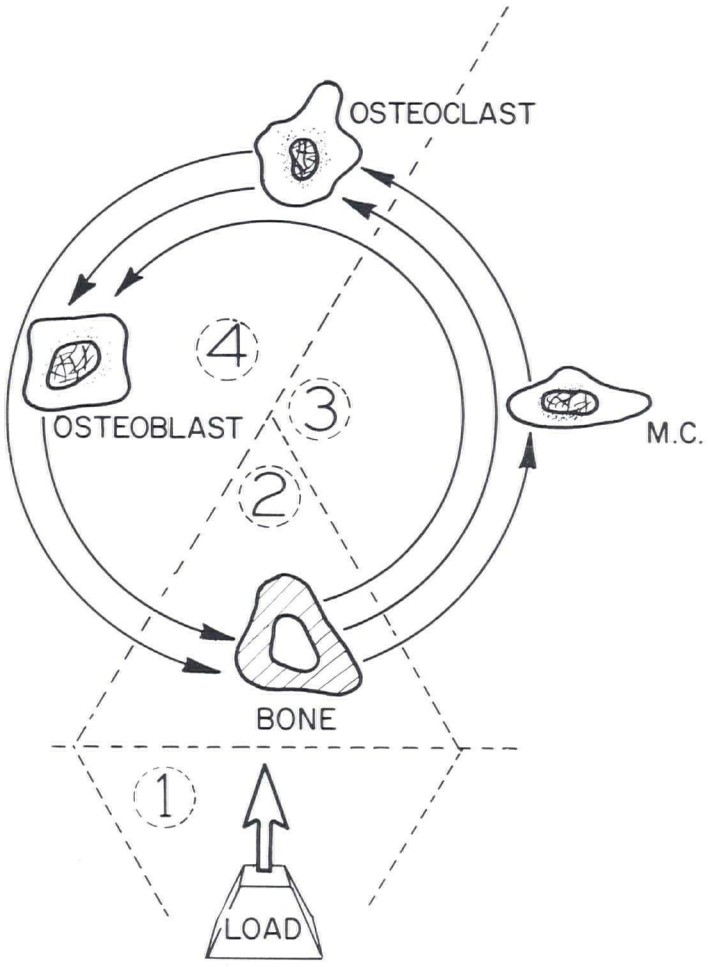

Figure 5

A diagram of the chain of causes or controls that converts muscle action on bones to bone size, shape and location in the limb. This is a set of interlocking negative feedback systems which is precise, and exquisite in its conception. (Reprinted by permission: Frost, H. M., The Laws of Bone Structure, Springfield, 1964.)

REFERENCES

1. Apter, M. J. and Wolpert, L.: Cybernetics and development. I. Information theory. J. Theor. Biol., 8:244-257, Mar. 1965.

2. Arnold, J. S.: "The quantitation of bone mineralization as an organ and tissue in osteoporosis," in Pearson, O. H., ed: *Dynamic Studies of Metabolic Bone Disease.* Philadephia, F. A. Davis Co., 1964.

3. Belanger, L. F. and Leblond, C. P.: Method for locating radioactive elements in tissues by covering histological sections with photographic emulsion. Endocrinology, 39:8-13, July 1946.

4. Duncan, A., Frost, H. M., Villanueva, A. R., Sigler, J.: The osteoporosis of rheumatoid arthritis. Arth. and Rheum. 8:943-954, Oct. 1965.

5. Enlow, D. H.: A plastic-seal method for mounting sections of ground bone. Stain Techn., 29:21-22, Jan. 1954.

6. Epker, B. N., Frost, H. M.: Correlation of patterns of bone resorption and formation with physical behavior of loaded bone. J. Dent. Res. 44:33, 1965.

7. Frost, H. M.: Preparation of thin, undecalcified bone sections by rapid manual method. Stain Techn., 33:273-277, Nov. 1958.

8. Frost, H. M.: A synchronous group of mammalian cells whose *in vivo* behavior can be studied. Henry Ford Hosp. Med. Bull., 13:161-172, June 1965.

9. Frost, H. M.: *The Laws of Bone Structure.* Springfield, Ill., Charles C. Thomas, 1964.

10. Frost, H. M.: *Mathematical Elements of Lamellar Bone Remodelling.* Springfield, Ill., Charles C. Thomas, 1964.

11. Hurxthal, L. M., Dotter, W. E., Baylink, D. J. and Clerkin, E. P.: Two new methods for the study of osteoporosis and other metabolic bone disease. Lahey Clin. Bull., 13:155-166, Jan.-Mar. 1964.

12. Jee, W. S. S. "The influence of reduced local vascularity on the rate of internal reconstruction in adult long bone cortex," in Frost, H. M., ed.: *Bone Biodynamics.* Henry Ford Hospital International Symposium, Boston, Little, Brown and Co., 1964.

13. Johnson, L. C.: "Morphologic analysis in pathology," in Frost, H. M., ed.: *Bone Biodynamics.* Henry Ford Hospital International Symposium, Boston, Little, Brown and Co., 1964.

14. Kelin, M. and Frost, H. M.: Lamellar bone physiology in diabetes mellitus: The numbers of bone resorption and formation foci in rib. Henry Ford Hosp. Med. Bull., 12:527-536, Dec. 1964.

15. Kember, N. F.: Cell division in endochondral ossification. J. Bone Joint Surg., 42B:824-839, Nov. 1960.

16. Klein, M., Villanueva, A. R., Frost, H. M.: A quantitative histological study of rib from 18 patients treated with adrenal cortical steroids. Acta Orthop. Scand., 35:171-184, 1965.

17. LeBond, C. P.: The time dimension in histology. Am. J. Anat., 116:1-27, Jan. 1965.

18. Milch, R. A., Rall, D. P. and Tobie, J. E.: Bone localization of the tetracyclines. J. Nat. Cancer Inst., 19:87-93, July 1957.

19. Owen M.: Cell population kinetics of an osteogenic tissue. I. J. Cell Biol.: 9:19-32, Oct. 1963.

20. Quastler, H. and Sherman, F. G.: Cell population kinetics in the intestinal epithelium of the mouse. Exp. Cell Res., 17:420-438, June 1959.

21. Takahashi, H., Epker, B., Frost, H. M.: Relation between age and size of osteons in man. Henry Ford Hosp. Med. Bull., 13:25-31, Mar. 1965.

22. Tonna, E. A.: The cellular complement of the skeletal system studied autoradiographically with tritiated thymidine (H3TDR) during growth and aging. J. Biophys. Biochem. Cytol., 9:813-824, Apr. 1961.

23. Villanueva, A. R., Hattner, R. S. and Frost, H. M.: A tetrachrome stain for fresh, mineralized bone sections, useful in the diagnosis of bone diseases. Stain Techn. 39:87-94, Mar. 1964.

24. Young, R. W.: "Specialization of bone cells, in Frost, H. M., ed. *Bone Biodynamics.* Henry Ford Hospital International Symposium, Boston, Little, Brown and Co., 1964.

25. "We" is neither editorial nor royal. It is the present and past members of the Laboratory: H. Roth, S. Stanisavljevic, B. Epker, R. Hattner, G. Scimeni, P. Santoro, A. Villanueva, M. Kelin, R. Ramser, H. Takahashi, L. Ilnicki, E. Sedlin.